(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
31 May 2001 (31.05.2001)

PCT

(10) International Publication Number
WO 01/37780 A2

(51) International Patent Classification[7]: A61K

(21) International Application Number: PCT/US00/32408

(22) International Filing Date:
29 November 2000 (29.11.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/168,141 29 November 1999 (29.11.1999) US

(71) Applicant *(for all designated States except US)*: SMITHKLINE BEECHAM CORPORATION [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: DHANAK, Dashyant [GB/US]; 1502 West Woodbank Way, West Chester, PA 19380 (US). KNIGHT, Steven, D. [US/US]; 115 Cromwell Lane, West Chester, PA 19380 (US). WARREN, Gregory, L. [US/US]; 1478 Old Schuylkill Road, Parkerford, PA 19457 (US).

(74) Agents: VENETIANER, Stephen et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

(81) Designated States *(national)*: CA, JP, US.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/37780 A2

(54) Title: UROTENSIN-II ANALOGS

(57) Abstract: The present invention relates generally Urotensin-II analogs and pharmaceutical compositions containing them.

UROTENSIN-II ANALOGS

FIELD OF THE INVENTION

The present invention relates generally Urotensin-II analogs and pharmaceutical compositions containing them.

BACKGROUND OF THE INVENTION

The integrated control of cardiovascular homeostasis is achieved through a combination of both direct neuronal control and systemic neurohormonal activation. Although the resultant release of both contractile and relaxant factors is normally under stringent regulation, an aberration in this *status quo* can result in cardiohemodynamic dysfunction with pathological consequences.

The principal mammalian vasoactive factors that comprise this neurohumoral axis, namely angiotensin-II, endothelin-1, norepinephrine, all function via an interaction with specific G-protein coupled receptors (GPCR). Urotensin-II, represents a novel member of this neurohumoral axis.

In the fish, this peptide has significant hemodynamic and endocrine actions in diverse end-organ systems and tissues:

- smooth muscle contraction

  both vascular and non-vascular in origin including smooth muscle preparations from the gastrointestinal tract, respiratory, and genitourinary tract. Both pressor and depressor activity has been described upon systemic administration of exogenous peptide

- osmoregulation:

  effects which include the modulation of transepithelial ion (Na^+, Cl^-) transport. Although a diuretic effect has been described, such an effect is postulated to be secondary to direct renovascular effects (elevated GFR)

- metabolism:

  urotensin-II influences prolactin secretion and exhibits a lipolytic effect in fish (activating triacylglycerol lipase resulting in the mobilization of non-esterified free fatty acids)

  (Pearson, *et. al. Proc. Natl. Acad. Sci. (U.S.A.)* **1980**, *77*, 5021; Conlon, *et. al. J. Exp. Zool.* **1996**, *275*, 226.)

In studies with human Urotensin-II it was found that it:

- was an extremely potent and efficacious vasoconstrictor

- exhibited sustained contractile activity that was extremely resistant to wash out
- had detrimental effects on cardiac performance (myocardial contractility)

Human Urotensin-II was assessed for contractile activity in the rat-isolated aorta and was shown to be the most potent contractile agonist identified to date. Based on the *in vitro* pharmacology and *in vivo* hemodynamic profile of human Urotensin-II it plays a pathological role in cardiovascular diseases characterized by excessive or abnormal vasoconstriction and myocardial dysfunction. (Ames *et. al. Nature* **1999**, *401*, 282)

Urotensin-II analogs are useful for identifying agonists and antagonists/inhibitors of urotensin II, and for treating conditions associated with Human Urotensin II imbalance. For example, facilitating the actions of the U-II system, either by mimicking the agonist activity of U-II at its cognate receptor(s) or by attenuating the uptake/metabolism of U-II.

These compounds may be useful in the treatment of congestive heart failure, stroke, ischemic heart disease (angina, myocardial ischemia), cardiac arrhythmia, hypertension (essential and pulmonary), COPD, restenosis, asthma, (Hay DWP, Luttmann MA, Douglas SA: 2000, Br J Pharmacol: volume 131, pages 10-12) neurogenic inflammation and metabolic vasculopathies all of which are characterized by abnormal vasoconstriction and/or myocardial dysfunction. Since U-II and GPR14 are both expressed within the mammalian CNS (Ames *et. al. Nature* **1999**, *401*, 282), they also may be useful in the treatment of addiction, schizophrenia, impulsivity, anxiety, stress, depression, and neuromuscular function. Functional U-II receptors are expressed in rhabdomyosarcomas cell lines and therefore may have oncological indications. Urotensin may also be implicated in various metabolic diseases such as diabetes (Ames *et. al. Nature* **1999**, *401*, 282, Nothacker et al., *Nature Cell Biology* 1: 383-385, 1999).

SUMMARY OF THE INVENTION

In one aspect this invention provides for urotensin II analogs and pharmaceutical compositions containing them.

In a second aspect, this invention provides for the use of urotensin analogs for identifying agonists and antagonists of urotensin II, and as inhibitors of urotensin II.

In another aspect, this invention provides for the use of urotensin analogs for treating conditions associated with urotensin II imbalance.

In and yet another aspect, this invention provides for the use of urotensin analogs for the treatment of congestive heart failure, stroke, ischemic heart disease (angina, myocardial ischemia), cardiac arrhythmia, hypertension (essential and pulmonary), COPD,

restenosis, asthma, neurogenic inflammation and metabolic vasculopathies, addiction, schizophrenia, impulsivity, anxiety, stress, depression, neuromuscular function, and diabetes.

Other aspects and advantages of the present invention are described further in the following detailed description of the preferred embodiments thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides for the following urotension II analogs and pharmaceutical compositions containing them :

H-Glu-Thr-Pro-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 1)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-OH (cyclic disulfide); (SEQ ID NO: 2)

Ac-Cys-Phe-Trp-Lys-Tyr-Cys-NH_2 (cyclic disulfide); (SEQ ID NO: 3)

H-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 4)

H-Asp-Cys-Cha-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 5)

H-Asp-Cys-Phe-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 6)

H-Asp-Cys-Phe-Trp-Lys-Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 7)

H-Asp-Cys-Cha-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 8)

H-Asp-Cys-Phe-Trp-Arg-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 9)

H-Asp-Cys-Phe-Trp-Orn-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 10)

H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 11)

H-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 12)

H-Gly-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 13)

H-Asp-Cys-(α-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 14)

H-Asp-Cys-(N-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 15)

H-Asp-Cys-(α-Me)Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 16)

H-Asp-Cys-Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 17)

H-Glu-Thr-Pro-Asp-Cys-Ala-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 18)

H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 19)

H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 20)

H-Arg-Arg-Arg-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 21)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Lys-OH (cyclic disulfide); (SEQ ID NO: 22)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asp-OH (cyclic disulfide); (SEQ ID NO: 23)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asn-OH (cyclic disulfide); (SEQ ID NO: 24)

H-Gln-Arg-Lys-Gln-His-Gly-Thr-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 25) and

H- Gln-His-Gly-Thr-Ala-Pro-Glu Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO:26)

or a pharmaceutically acceptable salt thereof.

The preferred compounds are:

H-Glu-Thr-Pro-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 1)

H-Asp-Cys-Phe-Trp-Lys-Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 7)

H-Asp-Cys-Phe-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 6)

H-Asp-Cys-Phe-Trp-Arg-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 9)

H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 11) and

H-Asp-Cys-(N-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide). (SEQ ID NO: 15)

The compounds of the present invention may contain one or more asymmetric carbon atoms and may exist in racemic and optically active form. All of these compounds and their diastereoisomers are contemplated to be within the scope of the present invention.

Preparation

Peptides were synthesized on *tert*-Butoxycarbonyl-L-cysteine(S-p-methoxybenzyl)-O-Resin (loading = 0.84 mole equiv/g). Preparation of the peptides consisted of the synthesis cycle, hydrofluoric acid cleavage, cyclization, and purification. All amino acids were used as alpha *tert*-butoxycarbonyl derivatives. Sidechain protecting groups were as follows:

Tryptophan	none
Tyrosine	Br-Z
Lysine	2-ClZ
Cysteine	p-methoxybenzyl
Aspartic acid	cyclohexyl

Experimental

Each synthesis cycle consisted of:

a) Trifluoroacetic acid deblock

The resin was treated with 50% TFA in methylene chloride (two to three times resin volume), stirred at room temperature for 30 min and drained. The resin was washed once with an equal volume of isopropanol for 1 min, then washed twice with an equal volume of methanol for 1 min.

b) Coupling

Resin from (a) above was washed with an equal volume of 10% triethylamine in methylene chloride twice for 1 min, then washed with an equal volume of methanol twice for 1 min, and finally washed with an equal volume of methylene chloride twice for 1 min. To the resin was added three equivalents of *tert*-butoxycarbonyl amino acid (dissolved in methylene chloride or methylene chloride/N,N-dimethylformamide mixture), three equivalents of 1-hydroxybenzotriazole hydrate (1 M solution in N,N-dimethylformamide) and the resultant suspension was stirred for one min. To the mixture was added three equivalents of dicyclohexylcarbodiimid (1 M solution in methylene chloride) and the reaction was stirred for 60-120 min. The resin was then washed with equal volume of methanol twice and washed with equal volume of methylene chloride twice. A small sample was taken for ninhydrin test: upon incomplete coupling, subcycle b) was repeated; upon complete coupling, the synthesis was continued with subcycle c).

c) Capping

To the resin from (b) above was added an equal volume of acetic anhydride (20% in methylene chloride) and the mixture was stirred for 5 min at room temperature. The resin was then washed with an equal volume of methanol twice and an equal volume of methylene chloride twice.

HF Cleavage:

To 1.0 g of resin from (c) above in a teflon reaction vessel was added 1 ml of anhydrous anisole. The vessel was cooled with liquid nitrogen and charged with 10 ml hydrofluoric acid (anhydrous, distilled). The temperature was raised with ice water to 0° C and the reaction stirred for 1 h. The hydrofluoric acid was distilled off at 0° C and the residue was

washed with anhydrous ether, extracted with 1:1 acetonitrile/water and lyophylized to furnish the cleaved linear peptide.

Cyclization:

After, the crude linear peptide was extracted from the cleaved resin, it was diluted to approx. 1 g/L with water. The pH was adjusted to 7.5 –8.0 with ammonium hydroxide. The cloudy reaction mixture was stirred until no more shift was detected by HPLC and/or the mixture become weak/negative by Ellman test (2-3 days). The mixture was acidified to pH 4.0 and filtered to provide the crude cyclic disulfide peptides.

Purification:

HPLC: column Vydac C-18 RP silica, 15-20 uM, 2" diameter

The crude peptides were loaded onto reverse phase HPLC column. A linear gradient was used over 30 min (100% water containing 0.1% trifluoroacetic acid to 80% acetonitrile containing 0.1% trifluoroacetic acid /20% water containing 0.1% trifluoroacetic acid). Fractions showing better than 95% purity were pooled and lyophylized to furnish the desired peptides.

Mass Spec. and Amino Acid Analysis were used to confirm the peptide sequence.

In order to use a compound of the Formula (I) or a pharmaceutically acceptable salt thereof for the treatment of humans and other mammals it is normally formulated in accordance with standard pharmaceutical practice as a pharmaceutical composition.

Compounds of Formula (I) and their pharmaceutically acceptable salts may be administered in a standard manner for the treatment of the indicated diseases, for example orally, parenterally, sub-lingually, transdermally, rectally, via inhalation or via buccal administration.

Compounds of Formula (I) and their pharmaceutically acceptable salts, which are active when given orally, can be formulated as syrups, tablets, capsules and lozenges. A syrup formulation will generally consist of a suspension or solution of the compound or salt in a liquid carrier for example, ethanol, peanut oil, olive oil, glycerin or water with a flavoring or coloring agent. Where the composition is in the form of a tablet, any pharmaceutical carrier routinely used for preparing solid formulations may be used. Examples of such carriers include magnesium stearate, terra alba, talc, gelatin, agar, pectin, acacia, stearic acid, starch, lactose and sucrose. Where the composition is in the form of a

capsule, any routine encapsulation is suitable, for example using the aforementioned carriers in a hard gelatin capsule shell. Where the composition is in the form of a soft gelatin shell capsule any pharmaceutical carrier routinely used for preparing dispersions or suspensions may be considered, for example aqueous gums, celluloses, silicates or oils and are incorporated in a soft gelatin capsule shell.

Typical parenteral compositions consist of a solution or suspension of the compound or salt in a sterile aqueous or non-aqueous carrier optionally containing a parenterally acceptable oil, for example polyethylene glycol, polyvinylpyrrolidone, lecithin, arachis oil, or sesame oil.

Typical compositions for inhalation are in the form of a solution, suspension or emulsion that may be administered as a dry powder or in the form of an aerosol using a conventional propellant such as dichlorodifluoromethane or trichlorofluoromethane.

A typical suppository formulation comprises a compound of Formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent, for example polymeric glycols, gelatins, cocoa-butter or other low melting vegetable waxes or fats or their synthetic analogues.

Typical transdermal formulations comprise a conventional aqueous or non-aqueous vehicle, for example a cream, ointment, lotion or paste or are in the form of a medicated plaster, patch or membrane.

Preferably the composition is in unit dosage form, for example a tablet, capsule or metered aerosol dose, so that the patients may administer to themselves a single dose.

No unacceptable toxicological effects are expected when compounds of the invention are administered in accordance with the present invention.

These urotensin analogs may be used for the treatment of congestive heart failure, stroke, ischemic heart disease (angina, myocardial ischemia), cardiac arrhythmia, hypertension (essential and pulmonary), COPD, restenosis, asthma, neurogenic inflammation and metabolic vasculopathies, addiction, schizophrenia, impulsivity, anxiety, stress, depression, neuromuscular function, and diabetes.

The biological activity of the compounds of Formula (I) are demonstrated by the following tests:

Radioligand binding:

HEK-293 cell membranes containing stable cloned human and rat GPR-14 (20 ug/assay) were incubated with 200 pM [125I] h-U-II (200 Ci/mmol^{-1} in the presence of increasing concentrations of test compounds in DMSO (0.1 nM to 10 uM), in a final

incubation volume of 200 ul (20 mM Tris-HCl, 5 mM MgCl2). Incubation was done for 30 minutes at room temperature followed by filtration GF/B filters with Brandel cell harvester. ^{125}I labeled U-II binding was quantitated by gamma counting. Nonspecific binding was defined by ^{125}I U-II binding in the presence of 100 nM of unlabeled human U-II. Analysis of the data was performed by nonlinear least square fitting.

Ca^{2+}-mobilization:

A microtitre plate based Ca^{2+}-mobilization FLIPR assay (Molecular Devices, Sunnyvale, CA) was used for the functional identification of the ligand activating HEK-293 cells expressing (stable) recombinant GPR-14. The day following transfection, cells were plated in a poly-D-lysine coated 96 well black/clear plates. After 18-24 hours the media was aspirated and Fluo 3AM-loaded cells were exposed to various concentrations (10 nM to 30 uM) of test compounds followed by h-U-II. After initiation of the assay, fluorescence was read every second for one minute and then every 3 seconds for the following one minute. The inhibitory concentration at 50% (IC50) was calculated for various test compounds.

Inositol phosphates assays:

HEK-293-GPR14 cells in T150 flask were prelabeled overnight with 1 uCi myo-[^3H] inositol per ml of inositol free Dulbecco's modified Eagel's medium. After labeling, the cells were washed twice with Dulbecco's phosphate-buffered saline (DPBS) and then incubated in DPBS containing 10 mM LiCl for 10 min at 37°C. The experiment was initiated by the addition of increasing concentrations of h-U-II (1 pM to 1μM) in the absence and presence of three different concentrations (0.3, 1 and 10 uM) of test compounds and the incubation continued for an additional 5 min at 37°C after which the reaction was terminated by the addition of 10% (final concentration) trichloroacetic acid and centrifugation. The supernatants were neutralized with 100ul of 1M Trizma base and the inositol phosphates were separated on AG 1-X8 columns (0.8 ml packed, 100-200 mesh) in formate phase. Inositol monophosphate was eluted with 8 ml of 200 mM ammonium formate. Combined inositol di and tris phosphate was eluted with 4ml of 1M ammonium formate/ 0.1 M formic acid. Eluted fractions were counted in beta scintillation counter. Based on shift from the control curve K_B was calculated.

Modulation of neuroendocrine factors

Adult Sprague Dawley rats are surgically prepared with guide cannulae directed towards the lateral ventricle (verified by an intense drinking response to angiotensin II; 100ng i.c.v.). Following a two week recovery period, rats receive human urotensin-II, putative agonist ligand (1-10 ug, i.c.v.) or vehicle (0.9% saline solution) over min (allowing 90 sec for diffusion) in order to detect any neuroendocrine plasma changes. Alternatively, anesthetized rats are prepared for acute systemic exposure to human urotensin-II, putative agonist ligand (100 ug, bolus i.v.) or vehicle (0.9% saline solution) via an i.v. cannula placed in the left femoral of jugular vein. After a period of 20 min, is blood collected for subsequent assay of the neuroendocrine markers using suitable radioimmunoassays (RIAs). Neuroendocrine markers include but are not limited to:

Pituitary hormones, both anterior (*e.g.* ADH, OCT, ACTH) and posterior (*e.g.* GH, TSH, LH, GSH, Prolactin)
Hypothalamic facors (SST, GHRH, TRH, CRH)
Thyroid/parathyroid hormones (T4, T3, rT3, calcitonin, PTH)
Insulin, leptin, glucose
Lipids
Sex hormones (including releasing hormones)
Vasoactive neurohormones (ET, Angiotensin II, aldosterone, NE)

Examples

Examples 1-26 were prepared following the general procedure outlined above using the appropriate starting materials:

Example	Peptide	MS (ES+) m/e
1	H-Glu-Thr-Pro-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 1)	1389, 1411
2	H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-OH (cyclic disulfide) (SEQ ID NO: 2)	962
3	Ac-Cys-Phe-Trp-Lys-Tyr-Cys-NH_2 (cyclic disulfide) (SEQ ID NO: 3)	888
4	H-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 4)	1062, 1084
5	H-Asp-Cys-Cha-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 5)	1058, 529
6	H-Asp-Cys-Phe-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 6)	1052, 526
7	H-Asp-Cys-Phe-Trp-Lys-Phe-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 7)	1046, 524
8	H-Asp-Cys-Cha-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 8)	1068, 535
9	H-Asp-Cys-Phe-Trp-Arg-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 9)	1090, 545
10	H-Asp-Cys-Phe-Trp-Orn-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 10)	1048, 1070
11	H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 11	1018
12	H-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 12)	1061, 1083
13	H-Gly-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 13)	1004, 1026
14	H-Asp-Cys-(α-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 14)	1075, 538
15	H-Asp-Cys-(N-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic	1076, 539

	disulfide) (SEQ ID NO: 15)	
16	H-Asp-Cys-(α-Me)Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 16)	1072
17	H-Asp-Cys-Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 17)	1058
18	H-Glu-Thr-Pro-Asp-Cys-Ala-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 18)	1312
19	H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 19)	1058, 706
20	H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 20)	1058, 706
21	H-Arg-Arg-Arg-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 21)	1291, 861
22	H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Lys-OH (cyclic disulfide) (SEQ ID NO: 22)	1091, 546
23	H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asp-OH (cyclic disulfide) (SEQ ID NO: 23)	1077, 539
24	H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asn-OH (cyclic disulfide) (SEQ ID NO: 24)	1077, 539
25	H-Gln-Arg-Lys-Gln-His-Gly-Thr-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 25)	694, 520
26	H- Gln-His-Gly-Thr-Ala-Pro-Glu Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide) (SEQ ID NO: 26)	834, 556

EXAMPLE 27

Formulations for pharmaceutical use incorporating compounds of the present invention can be prepared in various forms and with numerous excipients. Examples of such formulations are given below.

Inhalant Formulation

A compound of Formula I, (1 mg to 100 mg) is aerosolized from a metered dose inhaler to deliver the desired amount of drug per use.

Tablets/Ingredients	Per Tablet
1.Active ingredient (Cpd of Form. I)	40 mg
2.Corn Starch	20 mg
3.Alginic acid	20 mg
4.Sodium Alginate	20 mg
5.Mg stearate	1.3 mg
	2.3 mg

Procedure for tablets:

Step 1:Blend ingredients No. 1, No. 2, No. 3 and No. 4 in a suitable mixer/blender.

Step 2:Add sufficient water portion-wise to the blend from Step 1 with careful mixing after each addition. Such additions of water and mixing until the mass is of a consistency to permit its conversion to wet granules.

Step :The wet mass is converted to granules by passing it through an oscillating granulator using a No. 8 mesh (2.38 mm) screen.

Step 4:The wet granules are then dried in an oven at 140°F (60°C) until dry.

Step 5:The dry granules are lubricated with ingredient No. 5.

Step 6:The lubricated granules are compressed on a suitable tablet press.

The above specification and Examples fully disclose how to make and use the compounds of the present invention. However, the present invention is not limited to the particular embodiments described hereinabove, but includes all modifications thereof within the scope of the following claims. The various references to journals, patents and other publications which are cited herein comprise the state of the art and are incorporated herein by reference as though fully set forth.

What is claimed is:

1. A compound selected from:

H-Glu-Thr-Pro-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 1)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-OH (cyclic disulfide); (SEQ ID NO: 2)

Ac-Cys-Phe-Trp-Lys-Tyr-Cys-NH_2 (cyclic disulfide); (SEQ ID NO: 3)

H-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 4)

H-Asp-Cys-Cha-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 5)

H-Asp-Cys-Phe-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 6)

H-Asp-Cys-Phe-Trp-Lys-Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 7)

H-Asp-Cys-Cha-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 8)

H-Asp-Cys-Phe-Trp-Arg-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 9)

H-Asp-Cys-Phe-Trp-Orn-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 10)

H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 11)

H-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 12)

H-Gly-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 13)

H-Asp-Cys-(α-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 14)

H-Asp-Cys-(N-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 15)

H-Asp-Cys-(α-Me)Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 16)

H-Asp-Cys-Phe-Trp-Lys-(α-Me)Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 17)

H-Glu-Thr-Pro-Asp-Cys-Ala-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 18)

H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 19)

H-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 20)

H-Arg-Arg-Arg-Leu-Ser-Leu-Ser-Gln-Ser-Lys-Val-Leu-Pro-Asn-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 21)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Lys-OH (cyclic disulfide); (SEQ ID NO: 22)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asp-OH (cyclic disulfide); (SEQ ID NO: 23)

H-Asp-Cys-Phe-Trp-Lys-Tyr-Cys-Asn-OH (cyclic disulfide); (SEQ ID NO: 24)

H-Gln-Arg-Lys-Gln-His-Gly-Thr-Ala-Pro-Glu-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 25) and

H- Gln-His-Gly-Thr-Ala-Pro-Glu Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO:26)
or a pharmaceutically acceptable salt thereof.

2. A compound of claim 1 selected from:
H-Glu-Thr-Pro-Asp-Cys-Phe-D-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 1)
H-Asp-Cys-Phe-Trp-Lys-Phe-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 7)
H-Asp-Cys-Phe-Trp-Lys-Cha-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 6)
H-Asp-Cys-Phe-Trp-Arg-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 9)
H-Ala-Cys-Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide); (SEQ ID NO: 11) and
H-Asp-Cys-(N-Me)Phe-Trp-Lys-Tyr-Cys-Val-OH (cyclic disulfide). (SEQ ID NO: 15)

3. A pharmaceutical composition comprising a compound of claim 1 and a pharmaceutically acceptable carrier.

4. A method for treating conditions associated with Human Urotensin II imbalance by administering to a subject in need thereof an effective amount of a compound of claim 1.

5. A method for treating stroke by adiministering to a subject in need thereof an effective amount of a compound of claim 1.

SEQUENCE LISTING

<110> DHANAK, Dashyant
KNIGHT, Steven D.
WARREN, Gregory L.

<120> Urotension II Analogs

<130> P51081

<140> unknown
<141> 2000-11-29

<150> 60/168,141
<151> 1999-11-29

<160> 26

<170> PastSEQ for Windows Version 3.0

<210> 1
<211> 11
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 1

Glu	Thr	Pro	Asp	Cys	Phe	Trp	Lys	Tyr	Cys	Val
1				5					10	

<210> 2
<211> 7
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 2
Asp Cys Phe Trp Lys Tyr Cys
1 5

<210> 3
<211> 6
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 3
Cys Phe Trp Lys Tyr Cys
1 5

<220>
<223> cyclic disulfide

<210> 4
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 4
Asp Cys Phe Trp Lys Tyr Cys Val
1 5

<210> 5
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 5
Asp Cys Cha Trp Lys Cys Cha Val
1 5

<210> 6
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 6
Asp Cys Phe Trp Lys Cha Cys Val
1 5

<210> 7
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 7
Asp Cys Phe Trp Lys Phe Cys Val
1 5

<210> 8
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 8
Asp Cys Cha Trp Lys Tyr Cys Val
1 5

<210> 9
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 9
Asp Cys Phe Trp Arg Tyr Cys Val
1 5

<210> 10
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 10
Asp Cys Phe Trp Orn Tyr Cys Val
1 5

<220>
<223> cyclic disulfide

<210> 11
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 11
Ala Cys Phe Trp Lys Tyr Cys Val
1 5

<210> 12
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 12
Asn Cys Phe Trp Lys Tyr Cys Val
1 5

<210> 13
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 13
Gly Cys Phe Trp Lys Tyr Cys Val
1 5

<210> 14
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 14
Asp Cys Phe Trp Lys Tyr Cys Val
1 5

<210> 15
<211> 9
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 15
Asp Cys Asn Phe Trp Lys Tyr Cys Val
1 5

<210> 16
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 16
Asp Cys Phe Trp Lys Phe Cys Val
1 5

<210> 17
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 17
Asp Cys Phe Trp Lys Phe Cys Val
1 5

<210> 18
<211> 11

<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 18
Glu Thr Pro Asp Cys Ala Trp Lys Tyr Cys Val
1 5 10

<210> 19
<211> 18
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 19
Leu Ser Leu Ser Gln Ser Lys Val Leu Pro Asp Cys Phe Trp Lys Tyr
1 5 10 15
Cys Val

<210> 20
<211> 18
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 20
Leu Ser Leu Ser Gln Ser Lys Val Leu Pro Asn Cys Phe Trp Lys Tyr
1 5 10 15
Cys Val

<220>

<223> cyclic disulfide

<210> 21
<211> 21
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 21
Arg Arg Arg Leu Ser Leu Ser Gln Ser Lys Val Leu Pro Asn Cys Phe
1 5 10 15
Trp Lys Tyr Cys Val
20

<210> 22
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 22
Asp Cys Phe Trp Lys Tyr Cys Lys
1 5

<210> 23
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 23
Asp Cys Phe Trp Lys Tyr Cys Asp
1 5

<210> 24
<211> 8
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 24
Asp Cys Phe Trp Lys Tyr Cys Asn
1 5

<210> 25
<211> 17
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 25
Gln Arg Lys Gln His Gly Thr Ala Pro Glu Cys Phe Trp Lys Tyr Cys
1 5 10 15
Val

<210> 26
<211> 14
<212> PRT
<213> artificial sequence

<220>
<223> cyclic disulfide

<400> 26
Gln His Gly Thr Ala Pro Glu Cys Phe Trp Lys Tyr Cys Val
1 5 10